

Antibiotics Modulate Vaccine-Induced Humoral Immune Response

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The effects of antibiotics on the antigen-specific humoral immune response are not known. Macrolides, tetracyclines, and beta-lactams are commonly prescribed antibiotics. The first two are known to have immunomodulatory activities. The effects of clarithromycin, doxycycline, and ampicillin on the primary and secondary antibody responses to tetanus toxoid, a pneumococcal polysaccharide vaccine, a hepatitis B virus surface antigen (HBsAg) vaccine, and live attenuated *Salmonella typhi* (Ty21a) were investigated using a mouse model. For the mice receiving the tetanus toxoid, the immunoglobulin M (IgM) level of the clarithromycin group at day 7 was significantly lower than the corresponding antibody level of the normal saline (NS) group. For the mice receiving the pneumococcal polysaccharide vaccine, the total antibody and IgM levels of the clarithromycin group and the IgM level of the doxycycline group at day 7 were significantly lower than the corresponding antibody levels of the ampicillin and NS groups. For the mice receiving the HBsAg vaccine, the IgM level of the doxycycline group at day 7 was significantly lower than the corresponding antibody levels of the clarithromycin and NS groups, while the IgM level of the clarithromycin group at day 28 was significantly lower than the corresponding antibody levels of the doxycycline, ampicillin, and NS groups. For the mice receiving all three vaccines, there were no statistically significant differences between any of the antibody levels of the ampicillin group and the corresponding antibody levels of the NS group. For the mice receiving Ty21a, the total antibody levels of the ampicillin group at days 7 and 21 were significantly higher than the corresponding antibody levels of the NS group. Moreover, the IgM levels of the clarithromycin, doxycycline, and ampicillin groups at days 7 and 21 were significantly higher than the corresponding antibody levels of the NS group. Furthermore, the total antibody level of the ampicillin group at day 21 was significantly higher than the corresponding antibody level of the doxycycline group. For all four vaccines, there were no statistically significant differences among the serum levels of interleukin-10 and gamma interferon for the mice treated with the various antibiotics. We conclude that clarithromycin and doxycycline, but not ampicillin, suppress the antibody responses of mice to T-cell-dependent and T-cell-independent antigens, whereas all three antibiotics enhance the antibody response to live attenuated mucosal bacterial vaccines.

Antibiotics are well-known to have effects on the immune system, as shown by *in vitro*, *ex vivo*, and *in vivo* animal experiments and clinical studies. Regarding macrophage-monocyte functions, *in vitro* experiments have shown that macrolides stimulate phagocytic chemotaxis (4), promote monocyte-to-macrophage differentiation (11), and increase the killing capacity of macrophages (6); tetracyclines inhibit phagocytic chemotaxis and granuloma formation (25). As for cytokines, macrolides inhibit interleukin-1 (IL-1) production by murine peritoneal macrophages (22) and suppress IL-2 production induced by mitogen-stimulated T cells (15), while tetracyclines inhibit IL-1 and tumor necrosis factor alpha (TNF- α) production by human macrophages (19). In regard to lymphocytes, macrolides suppress mixed lymphocyte proliferation and the proliferative response of human peripheral blood mononuclear cells stimulated by polyclonal T-cell mitogens (15). Additionally, tetracyclines can protect mice from lethal endotoxemia (13), and we have recently shown that clarithromycin attenuates the surgical-trauma-induced inflammatory response in guinea pigs (26) and cyclophosphamide-induced mucositis in mice (27). In clinical studies, it has been shown that eryth-

romycin has an anti-inflammatory effect on patients with diffuse panbronchiolitis (17). Despite these findings, most of the experimental data to date relate to how antibiotics affect the innate immune response, cytokine levels, or nonspecific monocyte or lymphocyte proliferation. It has never been shown quantitatively how these antibiotics affect the effector arms of adaptive immunity, namely specific-antigen-induced antibody production and specific-antigen-induced lymphocyte proliferation or epitope-specific cytotoxic T-cell responses. The only study of antibody production and allograft rejection was not antigen specific (2).

Tetanus toxoid, pneumococcal polysaccharide vaccine, hepatitis B virus surface antigen (HBsAg) vaccine, and live attenuated *Salmonella typhi* are the prototypes of T-cell-dependent inactivated toxin, T-cell-independent polysaccharide, recombinant protein, and live attenuated vaccines, respectively. Their protective efficacies are often associated with the induction of antibody production in the host (3, 8, 10, 16, 21, 24). Since antibiotics of the macrolide, tetracycline, and penicillin groups are commonly prescribed and some of them have known effects on the immune system, but minor ailments such as upper respiratory tract infections may require antibiotic treatment and such treatment is not a known contraindication to vaccination, it is important to know whether antibiotics have any effects on the efficacy of immunization. In these experiments, we investigated the effect of clarithromycin (a commonly pre-

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scribed macrolide), doxycycline (a commonly prescribed tetracycline), and ampicillin (a commonly prescribed penicillin without a known effect on the immune system) on antibody production after tetanus toxoid, pneumococcal polysaccharide vaccine, HBsAg vaccine, and live attenuated *S. typhi* (Ty21a) administration to mice.

MATERIALS AND METHODS

Animals. Female BALB/c mice (18 to 22 g) were used in all experiments. They were housed in cages, each containing 10 mice, under standard conditions with regulated day length, temperature, and humidity, and they were given pelleted food and tap water *ad libitum*.

Immunization. On day zero, groups of 40 mice were immunized subcutaneously with tetanus toxoid with alum adjuvant (Berns, Bern, Switzerland; 2 limit flocculations (Lf) per mouse), subcutaneously with a pneumococcal polysaccharide vaccine (Pneumovax 23; Merck, Rahway, N.J.; 0.5 µg of each polysaccharide antigen per mouse), intraperitoneally with an HBsAg vaccine with alum adjuvant (HB-VAX II; MSD, Whitehouse Station, N.J.; 0.5 µg per mouse), or intraperitoneally with live attenuated *S. typhi* (Ty21a; Berns; 10^6 CFU per mouse) transformed with pBR322 (Amersham Pharmacia Biotech, Piscataway, N.J.) by electroporation (so as to make it ampicillin and tetracycline resistant [it is intrinsically resistant to clarithromycin]). On day 21, the same amount of tetanus toxoid, pneumococcal polysaccharide vaccine, or HBsAg vaccine was given to each member of the corresponding group of mice as a booster dose.

Administration of antibiotics. Clarithromycin (50 mg/kg), doxycycline (1.5 mg/kg), ampicillin (20 mg/kg), or normal saline (NS) (0.25 ml) was administered intraperitoneally to the mice of each group daily from day 1 prior to immunization (day -1) to day 27 postimmunization for the tetanus toxoid, pneumococcal polysaccharide vaccine, and HBsAg groups or to day 20 postimmunization for the Ty21a groups.

Measurement of antibody response. The mice were bled on days -1, 7, 21, and 28 for the tetanus toxoid, pneumococcal polysaccharide vaccine, and HBsAg groups and on days -1, 7, and 21 for the Ty21a groups. On days -1, 7, and 21, blood was taken just prior to administration of antibiotics. The blood was centrifuged at 2,700 × g for 20 min, and the supernatant (serum) was aliquoted and stored at -70°C until antibody measurements were performed.

Nuno-Immuno plates (Nalge Nunc International, Roskilde, Denmark) were used in all enzyme-linked immunosorbent assay (ELISA) experiments for measurement of antibody levels against tetanus toxoid, pneumococcal polysaccharide, and lipopolysaccharide of *S. typhi*. Each well was coated with 100 µl of diluted antigen (50 µl of tetanus toxoid in 50 µl of 0.05 M carbonate-bicarbonate buffer [pH 9.6], 0.1 µl of pneumococcal polysaccharide in 99.9 µl of phosphate-buffered saline [PBS], or 4 µg lipopolysaccharide of *S. typhi* in 0.05 M carbonate-bicarbonate buffer [pH 9.6]), and the plates were incubated at 4°C overnight. After the plates were washed with PBS-0.05% Tween 20 (washing buffer) twice, 200 µl of PBS-5% bovine serum albumin (BSA) (blocking buffer) was added to each well; the plates were then incubated at 37°C for 2 h. After the ELISA plates were washed with washing buffer three times, mouse sera (diluted with PBS-2% BSA) were added to them. For measurement of antibody levels against HBsAg, mouse sera (diluted with PBS-2% BSA) were added to ELISA plates precoated with HBsAg (Biotek, Barcelona, Spain). The plates were incubated at 37°C for 1 h. After the plates were washed with washing buffer three times, 100 µl of peroxidase-conjugated goat anti-mouse antibody (Serotec, Kidlington, United Kingdom), diluted with PBS-2% BSA according to the manufacturer's instructions, was added to each well; the plates were then incubated at 37°C for 30 min (tetanus toxoid, pneumococcal polysaccharide, and HBsAg) or 1 h (Ty21a). Immunoglobulin M (IgM) and total antibody levels were assayed to assess the primary and secondary immune responses, while IgG1 and IgG2a were measured to determine whether the humoral response had a more Th2-like or Th1-like pattern, respectively. After the plates were again washed with washing buffer three times, 100 µl of *ortho*-phenylenediamine (OPD) substrate (prepared by diluting 2 mg of OPD [Calbiochem, La Jolla, Calif.] in 2.5 ml of 50 mM citric acid [pH 5] with 2.5 µl of 30% H₂O₂) was added to each well; the plates were then incubated at room temperature for 30 min. A 100-µl aliquot of 1 M H₂SO₄ was added to each well, and the absorbance of each well was measured at 492 nm, using OPD buffer as a blank. Each sample was tested in duplicate, and the mean absorbance for each serum was calculated. All ELISAs were optimized so that there was a linear relationship between the optical density and the amount of antibody present in the serum at the serum dilution for the corresponding type of antibody measured. The serum antibody level of a particular mouse on a particular day was defined as the absorbance obtained from the serum on that day minus that of the same mouse on day -1. Control experiments were performed by adding ampicillin, clarithromycin, or doxycycline to serum samples so as to exclude the possibility of antibiotics interfering with the ELISA.

Measurement of serum levels of IL-10 and IFN-γ. Serum IL-10 and gamma interferon (IFN-γ) were measured by using commercial kits (Amersham Pharmacia, Little Chalfont, United Kingdom) to determine whether the immune response was more Th2 or Th1, respectively. Briefly, 50 µl of serum from each sample was added to the wells of ELISA plates precoated with monoclonal

antibodies against IL-10 or IFN-γ. The plates were incubated at 25°C for 3 and 2 h, respectively. For IL-10, the plates were washed with washing buffer three times, 50 µl of biotinylated antibody against IL-10 was added to each well, and the plates were then incubated at 25°C for 1 h. After the plates were again washed with washing buffer three times, 100 µl of streptavidin-horseradish peroxidase conjugate was added to each well prior to incubation at 25°C for 30 min. For IFN-γ, 100 µl of horseradish peroxidase-conjugated antibody against IFN-γ was added to each well. After the plates were washed with washing buffer three times, 100 µl of 3,3'-5,5'-tetramethylbenzidine substrate was added to each well, and the plates were incubated at room temperature for 30 min. Then 100 µl of 1 M H₂SO₄ was added per well, and the absorbance of each well was measured at 450 nm. The IL-10 and IFN-γ concentrations of individual samples were calculated by using standard curves prepared by performing the ELISA with known concentrations of the cytokines. The serum IL-10 or IFN-γ level of a particular mouse on a particular day is defined as the concentration of the cytokine on that day minus that of the same mouse on day -1.

Statistical analysis. Comparisons of the antibody and cytokine levels of mice in the clarithromycin, doxycycline, ampicillin, and NS groups receiving tetanus toxoid, pneumococcal polysaccharide vaccine, recombinant HBsAg vaccine, or Ty21a transformed with pBR322 were made by using Tukey's honestly significant difference test. A *P* < 0.05 is regarded as statistically significant.

RESULTS

The antibody levels at days 7, 21, and 28 after subcutaneous tetanus toxoid, subcutaneous pneumococcal polysaccharide vaccine, or intraperitoneal HBsAg vaccine administration to mice treated with clarithromycin, doxycycline, ampicillin, or NS are shown in Tables 1, 2, and 3, respectively. No effect of chemical interference of antibiotics on the ELISA was found, and there were no statistically significant differences among the antibody levels in the various groups of mice at day -1. For the mice receiving tetanus toxoid, the IgM level of the clarithromycin group at day 7 was significantly lower than the corresponding antibody level of the NS group. For the mice receiving the pneumococcal polysaccharide vaccine, the total antibody and IgM levels of the clarithromycin group and the IgM level of the doxycycline group at day 7 were significantly lower than the corresponding antibody levels of the ampicillin and NS groups. For the mice receiving the HBsAg vaccine, the IgM level of the doxycycline group at day 7 was significantly lower than the corresponding antibody levels of the clarithromycin and NS groups, while the IgM level of the clarithromycin group at day 28 was significantly lower than the corresponding antibody levels of the doxycycline, ampicillin, and NS groups. For the mice receiving all three of the vaccines, there were no statistically significant differences between the antibody levels of the ampicillin group and the corresponding antibody levels of the NS group.

The antibody levels at days 7 and 21 after intraperitoneal Ty21a administration to mice treated with clarithromycin, doxycycline, ampicillin, or NS are shown in Table 4. There were no statistically significant differences among the antibody levels in the various groups of mice at day -1. The total antibody levels of the ampicillin group at days 7 and 21 were significantly higher than the corresponding antibody levels of the NS group. Moreover, the IgM levels of the clarithromycin, doxycycline, and ampicillin groups at days 7 and 21 were significantly higher than the corresponding antibody levels of the NS group. Furthermore, the total antibody level of the ampicillin group at day 21 was significantly higher than the corresponding antibody level of the doxycycline group.

The serum IL-10 and IFN-γ levels of the mice administered the various vaccines and antibiotics are shown in Tables 5 and 6, respectively. For all four vaccines, there were no statistically significant differences among the IL-10 and IFN-γ levels of the mice administered the various antibiotics.

TABLE 1. Total antibody and antibody subtype levels at days 7, 21, and 28 after subcutaneous tetanus toxoid administration to mice treated with clarithromycin, doxycycline, ampicillin, or NS

| Day postvaccination | Antibody subtype | Serum dilution | Antibody level (A_{492}) | | | | SEM (pooled) | Critical value |
|---------------------|------------------|----------------|--------------------------------|-----------------------------|----------------------------|--------------------|--------------|----------------|
| | | | Mean for group treated with: | | | | | |
| | | | Clarithromycin ($n = 10$) | Doxycycline ($n = 10$) | Ampicillin ($n = 10$) | NS ($n = 10$) | | |
| 7 | Total | 1:500 | 0.150 | 0.122 | 0.152 | 0.164 | 0.015 | 0.058 |
| | IgM | 1:100 | 0.417 ^a | 0.554 | 0.659 | 0.696 ^a | 0.065 | 0.247 |
| | IgG1 | 1:50 | 0.265 | 0.216 | 0.186 | 0.173 | 0.048 | 0.182 |
| | IgG2a | 1:50 | 0.014 | 0.009 | 0.010 | 0.012 | 0.002 | 0.009 |
| 21 | Total | 1:5,000 | 0.484 | 0.486 | 0.480 | 0.472 | 0.044 | 0.168 |
| | IgM | 1:100 | 0.122 | 0.181 | 0.156 | 0.130 | 0.023 | 0.088 |
| | IgG1 | 1:500 | 0.382 | 0.354 | 0.364 | 0.355 | 0.019 | 0.071 |
| | IgG2a | 1:50 | 0.020 | 0.040 | 0.032 | 0.023 | 0.007 | 0.027 |
| 28 | Total | 1:50,000 | 0.200 | 0.174 | 0.185 | 0.158 | 0.016 | 0.060 |
| | IgM | 1:100 | 0.191 | 0.207 | 0.196 | 0.151 | 0.024 | 0.091 |
| | IgG1 | 1:5,000 | 0.286 | 0.217 | 0.235 | 0.243 | 0.025 | 0.096 |
| | IgG2a | 1:50 | 0.105 | 0.293 | 0.200 | 0.133 | 0.057 | 0.217 |

^a The difference is statistically significant compared to the mean with the same superscript.

DISCUSSION

This is the first study undertaken to show the effects of antibiotics on the B-cell response induced by specific antigens in a series of common vaccines. These vaccines were chosen because they represent prototypes of T-cell-dependent inactivated toxin, T-cell-independent polysaccharide, recombinant protein, and live attenuated vaccines against bacteria and viruses; clarithromycin, doxycycline, and ampicillin were chosen because they are commonly prescribed for minor ailments such as upper respiratory tract infection and acne vulgaris, and doxycycline and clarithromycin are known to have immunomodulating activities.

It has been known for a long time that antibiotics have various effects on the immune system (18). A number of groups have reported immunomodulatory effects of the macrolides and tetracyclines in vitro. The macrolides roxithromycin and erythromycin enhanced the phagocytosis of ³H-labelled *Staphylococcus aureus* by human macrophages (4) and increased the killing capacity for human macrophage-ingested live *Staphylococcus aureus* (6). Clarithromycin significantly inhibited IL production by murine peritoneal macrophages (22). Erythro-

mycin significantly increased the number of adherent human macrophages derived from monocytes after 7 days of culture (11). At concentrations of 40 to 200 µg/ml, midecamycin, josamycin, and clarithromycin suppressed the proliferative response of human peripheral blood mononuclear cells stimulated by polyclonal T-cell mitogens, and they also suppressed IL-2 production induced by mitogen-stimulated T cells at concentrations between 1.6 and 40 µg/ml (15). The combination of erythromycin and granulocyte-macrophage colony-stimulating factor and macrophage colony-stimulating factor additively and synergistically increased the number of monocyte-derived macrophages (11). The expression of surface antigen CD71, a macrophage activation marker, was increased when human macrophages were cultured in the presence of erythromycin (11). Recently, it was also reported that erythromycin ameliorated some chronic inflammatory processes of the respiratory tract, such as diffuse panbronchiolitis (17) and bronchial asthma (14), irrespective of its antibacterial properties. In one study of patients with panbronchiolitis, it was shown that erythromycin improved respiratory function and arterial blood gas tension irrespective of the presence of *Pseudomonas aerugi-*

TABLE 2. Total antibody and antibody subtype levels at days 7, 21, and 28 after subcutaneous pneumococcal polysaccharide vaccine administration to mice treated with clarithromycin, doxycycline, ampicillin, or NS

| Day postvaccination | Antibody subtype | Serum dilution | Antibody level (A_{492}) | | | | SEM (pooled) | Critical value |
|---------------------|------------------|----------------|--------------------------------|-----------------------------|----------------------------|----------------------|--------------|----------------|
| | | | Mean for group treated with: | | | | | |
| | | | Clarithromycin ($n = 10$) | Doxycycline ($n = 10$) | Ampicillin ($n = 10$) | NS ($n = 10$) | | |
| 7 | Total | 1:1,000 | 0.149 ^{a,b} | 0.217 | 0.281 ^a | 0.283 ^b | 0.031 | 0.118 |
| | IgM | 1:100 | 0.671 ^{c,d} | 0.673 ^{c,f} | 0.812 ^{c,e} | 0.807 ^{a,f} | 0.019 | 0.073 |
| | IgG1 | 1:50 | 0.017 | 0.014 | 0.016 | 0.011 | 0.002 | 0.009 |
| | IgG2a | 1:50 | 0.012 | 0.011 | 0.010 | 0.010 | 0.001 | 0.004 |
| 21 | Total | 1:1,000 | 0.343 | 0.361 | 0.345 | 0.347 | 0.029 | 0.110 |
| | IgM | 1:1,000 | 0.442 | 0.477 | 0.452 | 0.430 | 0.020 | 0.077 |
| | IgG1 | 1:50 | 0.017 | 0.011 | 0.016 | 0.019 | 0.004 | 0.014 |
| | IgG2a | 1:50 | 0.018 | 0.015 | 0.018 | 0.018 | 0.004 | 0.015 |
| 28 | Total | 1:1,000 | 0.277 | 0.298 | 0.284 | 0.277 | 0.034 | 0.128 |
| | IgM | 1:1,000 | 0.214 | 0.312 | 0.234 | 0.246 | 0.027 | 0.102 |
| | IgG1 | 1:50 | 0.016 | 0.012 | 0.019 | 0.027 | 0.007 | 0.025 |
| | IgG2a | 1:50 | 0.020 | 0.031 | 0.022 | 0.021 | 0.009 | 0.036 |

^{a-f} The difference is statistically significant compared to the mean with the same superscript.

TABLE 3. Total antibody and antibody subtype levels at days 7, 21, and 28 after intraperitoneal HBsAg vaccine administration to mice treated with clarithromycin, doxycycline, ampicillin, or NS

| Day postvaccination | Antibody subtype | Serum dilution | Antibody level (A_{492}) | | | | SEM (pooled) | Critical value |
|---------------------|------------------|----------------|--------------------------------|-----------------------------|----------------------------|--------------------|--------------|----------------|
| | | | Mean for group treated with: | | | | | |
| | | | Clarithromycin ($n = 10$) | Doxycycline ($n = 10$) | Ampicillin ($n = 10$) | NS ($n = 10$) | | |
| 7 | Total | 1:100 | 0.203 | 0.212 | 0.200 | 0.193 | 0.016 | 0.060 |
| | IgM | 1:100 | 0.357 ^a | 0.103 ^{a,b} | 0.201 | 0.322 ^b | 0.029 | 0.210 |
| | IgG1 | 1:50 | 0.027 | 0.026 | 0.026 | 0.027 | 0.002 | 0.007 |
| | IgG2a | 1:50 | 0.022 | 0.020 | 0.020 | 0.021 | 0.002 | 0.008 |
| 21 | Total | 1:100 | 0.293 | 0.466 | 0.506 | 0.721 | 0.143 | 0.543 |
| | IgM | 1:100 | 0.382 | 0.598 | 0.502 | 0.560 | 0.064 | 0.245 |
| | IgG1 | 1:50 | 0.049 | 0.069 | 0.123 | 0.222 | 0.060 | 0.229 |
| | IgG2a | 1:50 | 0.060 | 0.039 | 0.099 | 0.162 | 0.045 | 0.172 |
| 28 | Total | 1:1,000 | 0.440 | 0.631 | 0.789 | 0.785 | 0.106 | 0.405 |
| | IgM | 1:100 | 0.379 ^{a,d,e} | 0.602 ^c | 0.565 ^d | 0.574 ^c | 0.041 | 0.158 |
| | IgG1 | 1:50 | 0.335 | 0.710 | 1.056 | 1.130 | 0.222 | 0.845 |
| | IgG2a | 1:50 | 0.464 | 0.339 | 0.565 | 0.626 | 0.140 | 0.533 |

^{a-e} The difference is statistically significant compared to the mean with the same superscript.

nosa in the sputum (7). As for the tetracyclines, tetracycline, doxycycline, and minocycline inhibited granuloma formation in vitro in a dose-dependent manner at concentrations between 10^{-4} and 10^{-6} mol/liter through their action on protein kinase C (25). Tetracycline suppressed the synthesis of TNF- α and IL-1 in human macrophages (19). Recently, it was also reported that doxycycline (1.5 mg/kg) was able to inhibit TNF- α , IL-1, and nitrate secretion in the blood, with a decrease in inducible nitric oxide synthase activity in the spleen and peritoneal cells in a mouse model (13).

The classic primary antibody responses induced by tetanus toxoid, pneumococcal polysaccharide vaccine, and hepatitis B virus vaccine were suppressed by clarithromycin and doxycycline, as evidenced by the IgM levels in the clarithromycin and doxycycline groups being statistically lower than those in the ampicillin and/or NS groups. We speculate that this is partly due to a suppression of the T cell-B cell interaction in the production of antibodies. This is in line with the evidence showing that both clarithromycin and doxycycline can inhibit IL production by T lymphocytes in vitro (15, 19, 22). This is partly analogous to the suppressive effect on vaccination of glucocorticosteroids, which are well-known to down-regulate the production of IL-1, TNF- α , granulocyte-macrophage colony-stimulating factor, IL-3, IL-4, IL-5, IL-8, and inducible nitric oxide synthase (1). However, this cannot fully explain the

phenomenon, since antibody production after pneumococcal polysaccharide vaccine administration was also suppressed by both clarithromycin and doxycycline and since the humoral response to pneumococcal polysaccharide vaccine is well-known to be T-cell independent. Other possible targets of action of clarithromycin and doxycycline include antigen presentation, costimulatory signals, and postreceptor events of B-cell activation. Further experiments need to be performed before the exact mechanism can be elucidated.

The suppression by clarithromycin of the antibody response induced by the hepatitis B virus vaccine is persistent, as shown by a persistent suppression of the IgM level at day 28. Moreover, clarithromycin also suppressed the level of IgG1 against HBsAg at day 28, although this did not reach statistical significance. These phenomena were not observed in mice immunized with tetanus toxoid or the pneumococcal polysaccharide vaccine. It would be of both interest and clinical significance to know whether clarithromycin would have the same effect on immunization in humans, especially for HBsAg vaccination. If this is the case, the administration of clarithromycin, like that of glucocorticosteroid (12), cyclosporin A (5), and cytotoxic drugs such as cyclophosphamide (23), would be relatively contraindicated when people receive vaccinations.

There is no conclusive evidence showing any inclination of the immune response toward type Th1 or Th2 in the clarithro-

TABLE 4. Total antibody and antibody subtype levels at days 7 and 21 after intraperitoneal Ty21a-pBR322 administration to mice treated with clarithromycin, doxycycline, ampicillin, or NS

| Day postvaccination | Antibody subtype | Serum dilution | Antibody level (A_{492}) | | | | SEM (pooled) | Critical value |
|---------------------|------------------|----------------|------------------------------|----------------------|----------------------|------------------------|--------------|----------------|
| | | | Mean for group treated with: | | | | | |
| | | | Clarithromycin (n = 10) | Doxycycline (n = 10) | Ampicillin (n = 10) | NS (n = 10) | | |
| 7 | Total | 1:25 | 0.096 | 0.100 | 0.132 ^a | 0.069 ^a | 0.110 | 0.043 |
| | IgM | 1:25 | 0.226 ^b | 0.232 ^c | 0.234 ^d | 0.122 ^{b,c,d} | 0.024 | 0.093 |
| | IgG1 | 1:25 | 0.012 | 0.013 | 0.011 | 0.013 | 0.001 | 0.004 |
| | IgG2a | 1:25 | 0.010 | 0.010 | 0.020 | 0.010 | 0.004 | 0.016 |
| 21 | Total | 1:25 | 0.681 | 0.523 ^c | 0.973 ^{c,f} | 0.302 ^f | 0.101 | 0.386 |
| | IgM | 1:25 | 0.482 ^e | 0.524 ^b | 0.472 ^b | 0.158 ^{b,h,j} | 0.043 | 0.162 |
| | IgG1 | 1:25 | 0.017 | 0.015 | 0.012 | 0.012 | 0.002 | 0.007 |
| | IgG2a | 1:25 | 0.019 | 0.017 | 0.019 | 0.017 | 0.004 | 0.016 |

^{a-j} The difference is statistically significant compared to the mean with the same superscript.

TABLE 5. Serum IL-10 levels after subcutaneous tetanus toxoid, subcutaneous pneumococcal polysaccharide vaccine, intraperitoneal HBsAg, or intraperitoneal Ty21a-pBR322 administration to mice treated with clarithromycin, doxycycline, ampicillin, or NS

| Vaccine | Day postvaccination | Serum IL-10 level (pg/ml) | | | | SEM (pooled) | Critical value |
|-----------------------------|---------------------|------------------------------|----------------------|---------------------|--------------|--------------|----------------|
| | | Mean for group treated with: | | | | | |
| | | Clarithromycin (n = 10) | Doxycycline (n = 10) | Ampicillin (n = 10) | NS (n = 10) | | |
| Tetanus toxoid | 7 | 18 | 23 | 21 | 17 | 9 | 33 |
| | 21 | Undetectable* | Undetectable | Undetectable | Undetectable | | |
| | 28 | 55 | 26 | 56 | 88 | 26 | 98 |
| Pneumococcal polysaccharide | 7 | 5 | 4 | 4 | 13 | 3 | 13 |
| | 21 | 137 | 32 | 63 | 27 | 44 | 167 |
| | 28 | 17 | 12 | 16 | 17 | 9 | 35 |
| HBsAg | 7 | 31 | 31 | 40 | 49 | 17 | 63 |
| | 21 | 15 | 35 | 35 | 40 | 15 | 58 |
| | 28 | 2 | 12 | 4 | 2 | 2 | 8 |
| Ty21a | 7 | Undetectable | Undetectable | Undetectable | Undetectable | | |
| | 21 | Undetectable | Undetectable | Undetectable | Undetectable | | |

^a Undetectable, no statistically significant difference between the serum IL-10 levels on that day and day -1.

mycin or doxycycline groups. Although clarithromycin, and to a lesser extent doxycycline, suppressed the level of IgG1 against HBsAg on days 21 and 28 (not statistically significant), no effect on this antibody subclass was found with respect to the other vaccines. Furthermore, for all four vaccines, no difference in the IL-10 or IFN- γ levels can be shown among the mice administered the various antibiotics.

Paradoxically, the antibody responses induced by Ty21a were enhanced by clarithromycin and doxycycline, despite the immunosuppressive effect of these two antibiotics. Furthermore, the antibody response was also enhanced by ampicillin, which is not known to have any immunomodulating effects and has been shown in this study not to affect the antibody response induced by tetanus toxoid, pneumococcal polysaccharide vaccine, or hepatitis B virus vaccine. There is evidence showing that the antibody response of mice against *Escherichia coli* and the protection against wild-type *E. coli* challenge can be augmented by culturing live attenuated *E. coli* in the presence of aztreonam before immunization. The author speculated that this might be due to the partial damage of the bacteria by a sublethal dose of aztreonam, rendering the organisms more immunogenic (9). In our experiments, daily administration of antibiotics to the mice could have also sublethally damaged the

Ty21a, making it more immunogenic and therefore inducing an enhanced antibody response. Moreover, the total antibody level of the ampicillin group on day 21 was the highest among all the groups, significantly higher than that of the doxycycline group. This can be explained by the absence of an immunosuppressive effect of ampicillin, such that the antibiotic's immunogenic effect acts on its own. Since the clinical efficacy of the Ty21a vaccine is only 70% in humans (20, 24), the present observation could be important for enhancing the efficacy of the vaccine.

In conclusion, clarithromycin and doxycycline suppress the antibody response induced by tetanus toxoid, pneumococcal polysaccharide vaccine, and HBsAg through their immunomodulating effects, while ampicillin, clarithromycin, and doxycycline enhance the antibody response induced by Ty21a. This may be due to the antibiotic's immunogenic effect, which may overwhelm the immunomodulating effect of clarithromycin and doxycycline. Although the exact mechanism of suppression and enhancement of the antibody response remains to be elucidated, the present observations should prompt further investigation of the practical significance of such phenomena in terms of clinical implications and applications.

TABLE 6. Serum IFN- γ levels after subcutaneous tetanus toxoid, subcutaneous pneumococcal polysaccharide vaccine, intraperitoneal HBsAg, or intraperitoneal Ty21a-pBR322 administration to mice treated with clarithromycin, doxycycline, ampicillin, or NS

| Vaccine | Day postvaccination | IFN- γ level (pg/ml) | | | | SEM (pooled) | Critical value |
|-----------------------------|---------------------|------------------------------|----------------------|---------------------|--------------|--------------|----------------|
| | | Mean for group treated with: | | | | | |
| | | Clarithromycin (n = 10) | Doxycycline (n = 10) | Ampicillin (n = 10) | NS (n = 10) | | |
| Tetanus toxoid | 7 | 135 | 180 | 134 | 240 | 76 | 289 |
| | 21 | 705 | 1,006 | 943 | 620 | 401 | 1,526 |
| | 28 | 956 | 350 | 390 | 480 | 242 | 923 |
| Pneumococcal polysaccharide | 7 | 769 | 654 | 1,047 | 1,600 | 534 | 2,035 |
| | 21 | Undetectable ^a | Undetectable | Undetectable | Undetectable | | |
| | 28 | Undetectable | Undetectable | Undetectable | Undetectable | | |
| HBsAg | 7 | 769 | 1,032 | 857 | 570 | 352 | 1,340 |
| | 21 | Undetectable | Undetectable | Undetectable | Undetectable | | |
| | 28 | 678 | 943 | 845 | 460 | 325 | 1,236 |
| Ty21a | 7 | 1,523 | 704 | 1,045 | 645 | 435 | 1,655 |
| | 21 | 310 | 212 | 1,201 | 625 | 261 | 995 |

^a Undetectable, no statistically significant difference between the serum IFN- γ levels on that day and day -1.

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REFERENCES

- Barnes, P. J., and I. Adcock. 1993. Anti-inflammatory actions of steroids: molecular mechanisms. *Trends Pharmacol. Sci.* 14:536-41.
- Bellahsene, A., and A. Forsgren. 1985. Effect of doxycycline on immune response in mice. *Infect. Immun.* 48:556-559.
- Blake, P. A., D. M. Musher, J. E. Groover, R. A. Feldman, and T. M. Buchanan. 1976. Serologic therapy of tetanus in the United States. *JAMA* 235:42-44.
- Carlone, N. A., A. M. Cuffini, V. Tullio, and D. Sassetta. 1989. Comparative effects of roxithromycin and erythromycin on cellular immune functions in vitro. 2. Chemotaxis and phagocytosis of ^3H -*Staphylococcus aureus* by human macrophages. *Microbios* 58:17-25.
- Clerici, M., and G. M. Shearer. 1990. Differential sensitivity of human T helper cell pathways by in vitro exposure to cyclosporin A. *J. Immunol.* 144:2480-2486.
- Cuffini, A. M., N. A. Carlone, V. Tullio, and M. Borsetto. 1989. Comparative effects of roxithromycin and erythromycin on cellular immune functions in vitro. 3. Killing of intracellular *Staphylococcus aureus* by human macrophages. *Microbios* 58:27-33.
- Fujii, T., J. Kadota, K. Kawakami, K. Iida, R. Shirai, M. Kaseda, S. Kawamoto, and S. Kohno. 1995. Long term effect of erythromycin therapy in patients with chronic *Pseudomonas aeruginosa* infection. *Thorax* 50:1246-1252.
- Germanier, R., and E. Furrer. 1975. Isolation and characterization of GalE mutant Ty21a of *Salmonella typhi*: a candidate strain for a live, oral typhoid vaccine. *J. Infect. Dis.* 131:553-558.
- Giammarco, R., M. T. Lun, G. Luorino, C. Nazzari, A. Gaeta, C. Mancini, and F. Filodoro. 1993. Reactivity and protective capacity of a polyclonal antiserum derived from mice immunized with antibiotic exposed *Escherichia coli*. *J. Antimicrob. Chemother.* 31:117-128.
- Hamill, R. J., D. M. Musher, J. E. Groover, P. J. Zavell, and D. A. Watson. 1992. IgG antibody reactive with five *Streptococcus pneumoniae* serotypes in commercial intravenous immunoglobulin preparations and relationship to mouse protection. *J. Infect. Dis.* 166:38-42.
- Kisho, N., S. Kudo, H. Yotsuoto, and S. Akagawa. 1994. Erythromycin promotes monocyte to macrophage differentiation. *J. Antibiot.* 47:80-89.
- Markham, R. B., P. W. Stashak, B. Prescott, D. F. Amisbaugh, and P. J. Baker. 1978. Selective sensitivity to hydrocortisone of regulatory functions that determine the magnitude of the antibody response to type III pneumococcal polysaccharide. *J. Immunol.* 121:829-834.
- Milano, S., F. Arcolio, P. D'Agostino, and E. Cillari. 1997. Intraperitoneal injection of tetracyclines protects mice from lethal endotoxemia downregulating inducible nitric oxide synthase in various organs and cytokine and nitrate secretion in blood. *Antimicrob. Agents Chemother.* 41:117-121.
- Miyatake, H., F. Taki, H. Taniguchi, R. Suzuki, K. Takagi, and T. Satake. 1991. Erythromycin reduces the severity of bronchial hyperresponsiveness in asthma. *Chest* 99:670-673.
- Morikawa, K., F. Oesko, S. Morikawa, and K. Iwamoto. 1994. Immunomodulatory effects of three macrolides, midecamycin acetate, josamycin, and clarithromycin, on human T-lymphocyte function in vitro. *Antimicrob. Agents Chemother.* 38:2643-2647.
- Musher, D. M., B. Johnson, Jr., and D. A. Watson. 1990. Quantitative relationship between antipneumococcal antibody measured by enzyme-linked immunosorbent assay or radioimmunoassay and protection of mice against challenge with *Streptococcus pneumoniae* serotype 4. *Infect. Immun.* 58:3871-3876.
- Nagai, H., H. Shishido, R. Yoneda, E. Yamaguchi, A. Tamura, and A. Kurashima. 1991. Long-term low-dose administration of erythromycin to patients with diffuse bronchiolitis. *Respiration* 58:145-149.
- Rits, R. E. 1990. Antibiotics as biological response modifiers. *J. Antimicrob. Chemother.* 26(Suppl. C):31-36.
- Shapira, L., W. A. Soskine, Y. Hourv, V. Barak, A. Halabi, and A. Stabbok. 1996. Protection against endotoxin shock and lipopolysaccharide-induced local inflammation by tetracycline: correlation with inhibition of cytokine secretion. *Infect. Immun.* 64:825-828.
- Sinanjung, C. H., F. P. Palacios, N. H. Punjabi, R. Darmowigoto, Soprawito, H. Totowidjo, P. Haryanto, E. Supriyanto, N. D. Witham, and S. L. Hoffman. 1991. Oral immunization against typhoid fever in Indonesia with Ty21a vaccine. *Lancet* 338:1055-1059.
- Szmuness, W., C. E. Stevens, and F. A. Zang. 1982. A controlled trial on the efficacy of the hepatitis B vaccine (Heptavax B): a final report. *Hepatology* 1:377-385.
- Takeshita, K., I. Yamagishi, and M. Harada. 1989. Immunological and anti-inflammatory effects of clarithromycin: inhibition of interleukin 1 production by murine peritoneal macrophages. *Drugs Exp. Clin. Res.* 15:527-533.
- Valtonen, M. V., and P. Hiyry. 1978. O antigen as virulence factor in mouse typhoid: effect of B-cell suppression. *Infect. Immun.* 19:26-28.
- Wahdan, M. H., C. Serie, Y. Cersier, S. Sallam, and R. Germanier. 1982. A controlled field trial of live *Salmonella typhi* strain Ty21a oral vaccine against typhoid: three year results. *J. Infect. Dis.* 145:292-295.
- Webster, G. F., S. M. Tose, and L. Hegemann. 1994. Inhibition of a model of in vitro granuloma formation by tetracyclines and ciprofloxacin. Involvement of protein kinase C. *Arch. Dermatol.* 130:748-752.
- Woo, P. C. Y., L. W. C. Chow, E. S. K. Ma, and K. Y. Yuen. 1999. Clarithromycin attenuates the inflammatory response induced by surgical trauma in a guinea pig model. *Pharmacol. Res.* 39:49-54.
- Woo, P. C. Y., W. F. Ng, H. C. H. Leung, H. W. Tsoi, K. Y. Yuen. Clarithromycin attenuates cyclophosphamide-induced mucositis in mice. *Pharmacol. Res.*, in press.